Research Article

DISTRIBUTION OF ABO BLOOD GROUPS AND ALLELE FREQUENCY IN MAJOR ETHNIC GROUPS OF DARJEELING AND KURSEONG HILLS, WEST BENGAL, INDIA

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ABSTRACT

Introduction: The aim of the this study was to determine the frequency of the ABO blood group among the major ethnic communities and to analyze the ethno-demographic profile of the Hill sub-communities of Darjeeling and Kurseong Hills of the State of West Bengal, India. **Materials and Methods**: Total of 9030 individuals, out of which 7124 and 1906 individuals were from Darjeeling and Kurseong respectively. The collection of ABO Blood group data was collected from Blood donation camps organized by Red Cross Society, Darjeeling, and collection of data was also done from the recorded data of District Hospital, Darjeeling and Primary Health care units in Darjeeling rural areas. Similarly collection of Blood group data was collected from Sub-Divisional Hospital, Kurseong and from the Blood donation camps organized by Red Cross Society, Kurseong. Further, blood group data of individuals were also collected from diagnostic laboratories. **Results**: In the present data the most frequent ABO blood group were A (37.34%) and O (30.37%) followed by B(22.66%), AB (9.61%)), The phenotypic frequency was in the order of A>O>B>AB. The allele frequency of the population understudy was found to be p (A)= 0.272; q(B)=0.178; r(O)=0.550 in the frequency order O>A>B. The allele frequency of blood group O (r) was found to be most prevalent. **Conclusion:** The ethnographic study provided an insight into the distribution pattern of ABO blood group and allele frequencies in major ethnic groups of Darjeeling and Kurseong Hills of West Bengal, India. Moreover the present data may also be useful in population genetics studies, population migration patterns, forensics and genetic studies and genetic counseling.

INTRODUCTION

ABO Blood group system was discovered by Karl Landsteiner and his students (Decastello and Sturli) in the early 1900s and later Bernstein in 1925 showed that the ABO blood group system consist of three alleles of a single gene namely I^A, I^B, and I^O forming four different groups ; A ($I^A I^A$ or $I^A I^O$), B ($I^B I^B$ or $I^B I^O$), AB ($I^A I^B$) and O ($I^O I^O$).More than 70 molecular alleles are known but most of them can be assigned to be one of the three broad classes known as I^A, I^B, I^O. (Yip, 2002). It is to be noted that only alleles have continuity over time and the gene pool evolves when allele frequencies change. Furthermore, there are always fewer alleles than genotypes. So, the population can be described with fewer parameters when allele frequencies can be used (Russel, 2006). Hardy-Weinberg Principle was first put to test in connection with the ABO blood groups, where multiple allelism was first demonstrated. Population genetics has made extensive use of the blood groups, the influence of selective drift and gene flow has been studied using these groups. Blood groups also illustrate the difficulties in distinguishing close linkage and allelism in man

(Mckusick, 1978).The practical implication of population genetics is to design studies, to sample, preserve and record genetic variation among human populations throughout the world.

Darjeeling and Kurseong hills of the state of West Bengal were chosen for blood sample collections of the various hill sub-communities to study the distribution and variation of ABO blood types in these populations. Further, a comparative analysis of populations variations, phenotypic frequencies and allele frequencies were studied with regards to the pattern of distribution of ABO blood types.

An ethnographic survey was made to study and analyze the ethno-demographic profile of the Hill sub-communities of Darjeeling and Kurseong. The multi-ethnic, multi-cultural and multi-lingual composition of the hill population provides us with an interesting insight into the prospect to delve into research problems such as ancestry, population migration and evolutionary changes that may have taken place in the course of long association of different ethnic groups. In addition to the importance of these ABO blood group analyses in blood

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transfusion, their usefulness in population genetics studies, population migration patterns, forensics and disputed paternity cases is well established.(Pennap, *et al* 2011; Mondal *et al*., 2012).These findings emphasizes on the hypothesis that individuals of different blood types have considerable physiological differences.(Miller, *et al*.,1979)

The present study was carried out to assess the distribution pattern of ABO blood groups covering 19 sub-communities of the Darjeeling and Kurseong hills of the state of West Bengal belonging to diverse ethnic groups. Furthermore only very limited study have been conducted to understand the distribution of ABO group frequency and allelic frequency among the diverse ethnic people residing the Hilly regions of Darjeeling and Kurseong.

MATERIALS AND METHODS

Subjects

This study was carried out on 9030 individuals, out of which 7124 from Darjeeling Hill region and 1906 individuals from Kurseong Hill region of the state of West Bengal. The sample included belonging to three major ethnic groups like Nepali/Gorkha, Lepcha and Bhutia. The Nepali/Gorkha community has been further divided into 17 sub-communities like Rai, Subba,Newar, Thapa (Mangar), Gurung, Tamang, Sherpa,Thami,Sharma(Bhahun), Chhetri, Sunwar,Thakuri, Kami, Damai, Sarki, Lama, Mukhia.

Collection of Blood Group data

The collection of ABO Blood group data was collected from Blood donation camps organized by Red Cross Society, Darjeeling, collection of data was also done from the recorded data of District Hospital, Darjeeling and Primary Health care units in Darjeeling rural areas. Similarly collection of Blood group data was collected from Sub-Divisional Hospital, Kurseong and from the Blood donation camps organized by Red Cross Society, Kurseong. Further, blood group data of individuals were also collected from diagnostic laboratories.

Statistical Analysis

The blood group frequency and percentage of each group A,B.AB,O from Darjeeling and Kurseong was calculated. The allele frequencies of blood group were calculated by using Hardy-Weinberg model. Allele frequency (p,q,r) from the data collected from Darjeeling and Kurseong were calculated under the assumption of Hardy-Weinberg equilibrium.

Table 1 Hardy-Weinberg model for ABO blood group

Phenotype (Blood group)	Genotype	Phenotypic Frequency	Genotypic Frequency	Expected Frequency
A	AA+AO	nA	nAA + nAO	$p^2 + 2pr$
B	BB+BO	nB	nBB + nBO	$q^2 + 2qr$
O	AB	nAB	nAB	$2pq$
AB	OO	nO	nOO	r^2

Allelic frequencies

Allelic frequencies were calculated under the standard assumption of Hardy Weinberg equilibrium with Ceppilini correction. This iterative method yields maximum likelihood estimates. It was assumed that: The ABO system is determined by three alleles of a single gene, call them A, B and O.

- A and B are co-dominant and both are dominant over O.
- This gene is in accordance to Hardy-Weinberg frequencies in the population.
- The data were a random sample from the population.
- Static allele frequencies in a population across generations assume: No mutation (the alleles don't change), no migration or emigration (no exchange of alleles between populations), infinite population size and no selective pressure for or against any genotypes.

Chi-square test for goodness of fit of observed and expected (using estimated values) phenotype numbers was also calculated.

RESULTS

The prevalence of the distribution of A,B,AB,O blood group was determined for total 9030 individuals, out of which 7124 and 1906 individuals were from Darjeeling and Kurseong respectively. In the present population under study the most frequent ABO blood group were A (37.34%) and O (30.37%) followed by B(22.66%), AB (9.61%)), The frequency was in the order of A>O>B>AB.

ABO frequency and allele frequency of major ethnic population was studied namely Lepcha, Bhutia and Nepali/Gorkha community. Among 206 individuals of Lepcha community the most frequent blood group were A (43.20%, n=89) and O (30.58%, n=63), followed by B (21.84%, n=45) and AB (4.36%,n= 09), frequency was in the order of A>O>B>AB. Similarly, among 160 individuals of Bhutia community the most frequent blood group were O (36.25%, n=58) and B (30.6%, n=49), followed by A (25.0%, n=40) and AB (8.12%,n= 13), frequency was in the order of O>B>A>AB. In Nepali/Gorkha community Group A (37.43%, n=3243) and O (30.26%, n=2622) followed by group B (22.54%, n=1953) and AB (9.76%, n=846), the frequency was in the order of A>O>B>AB. (Table 2)

The allelic frequency (p, q, r) of all ethnic groups were calculated and the results compared between different sub-communities .The frequencies of the I^A (p), I^B (q) and I^O (r) alleles were tested according to the Hardy Weinberg law of Equilibrium..(Table 4).

The allelic frequency of the total population under study was calculated to be, p (A)= 0.2717; q(B)=0.1772; r(O)=0.5511.In the frequency order O>A>B. The Chi-square test for goodness of fit between the observed and expected phenotypes in case of ABO group was not found to be statistically significant (*P* > 0.0001) as evident from (Table 3)

Table 2 Distribution of ABO blood group in major communities of Darjeeling and kurseong

	Number of Individuals					
				Major communities		
Blood Group	Total Number of Individuals	Darjeeling	Kurseong	Lepcha	Bhutia	Nepali/Gorkha
A	3372 (37.34%)	2747 (38.55%)	625 (32.79%)	89 (43.2%)	40 (25.0%)	3243 (37.43%)
B	2047 (22.66%)	1568 (22.01%)	479 (25.13%)	45 (21.84%)	49 (30.6%)	1953 (22.54%)
AB	868 (9.61%)	696 (9.76%)	172 (9.02%)	09 (4.36%)	13 (8.12%)	846 (9.76%)
O	2743 (30.37%)	2113 (29.66%)	630 (33.05%)	63 (30.58%)	58 (36.25%)	2622 (30.26%)
Total	9030	7124	1906	206	160	8664

Table 3 Distribution of ABO frequency in total sample population under study from Darjeeling and Kurseong Hills of the State of West Bengal

Blood Group ABO	Phenotype	Observed Frequency	Genotype			Expected Frequency
A-Type	A	0.3734	AA	p^2	0.0739	0.3733
			AO	2pr	0.2994	
B-Type	B	0.2267	BB	q^2	0.0313	0.2266
			BO	2qr	0.1953	
O- Type	O	0.3038	OO	r^2	0.3038	0.3038
AB-Type	AB	0.0961	AB	2pq	0.0963	0.0963
Total		1			1	1

Allele Frequency (p, q, r) calculation of the total sample population under study in Darjeeling and Kurseong

Calculation of O allele frequency I^O(r)

Frequency of allele O is equal to the frequency of recessive genotype r^2.

r^2 = frequency of O phenotype (No individuals of O blood type/Total No of individuals)(O/N)

r^2= O/N

r = √O/N

r = √0.3734   r = 0.5511

Calculation of A allele frequency I^A (p)

p= frequency of the A phenotype + frequency of the O phenotype

The frequency of A+O = p^2+2pr+r^2 and   [$(p+r)^2$= p^2+2pr+r^2]

A+O= $(p+r)^2$

$(p+r)^2$ = A+O

p+r = √ A+O

p= √ A+O - r

p = √ 0.3734+0.3038 - 0.5511

p = 0.2717

Calculation of B allele frequency I^B (q)

Since (p+q+r=1)

q=1- (p+r)

q = 1 - (0.2717 + 0.5511)

q = 0.1771

The Lepcha and Bhutia community has allele frequencies p (A)= 0.3059; q(B)=0.1412; r(O)=0.5529 and p (A)= 0.1806; q(B)=0.2173; r(O)=0.6020 respectively.(Table 4)

The Nepali/Gorkha community comprises of 17 sub-communities which are multi-ethnic, multi-lingual and multi-cultural.

Table 4 Distribution of ABO Blood group and calculation of Allele frequency estimate of sub-communities of hill population of Darjeeling and Kurseong, West Bengal

Sub-Community	Allele Frequency					
	(p)		(q)		(r)	
Types	Darjeeling	Kurseong	Darjeeling	Kurseong	Darjeeling	Kurseong
Bhujel	0.307	0.22	0.173	0.18	0.520	0.60
Chhetri	0.263	0.22	0.193	0.20	0.544	0.58
Damai	0.188	0.153	0.240	0.288	0.572	0.559
Gurung	0.178	0.228	0.196	0.265	0.626	0.539
Kami	0.218	0.20	0.198	0.227	0.590	0.573
Lama	0.187	0.139	0.174	0.211	0.639	0.65
Mukhia	0.397	0.335	0.147	0.186	0.456	0.479
Tamang	0.227	0.185	0.172	0.171	0.601	0.644
Thakuri	0.416	0.108	0.146	0.185	0.438	0.707
Pradhan	0.319	0.312	0.207	0.151	0.474	0.537
Rai	0.384	0.31	0.133	0.190	0.483	0.50
Thapa	0.281	0.24	0.199	0.18	0.520	0.58
Sarki	0.104	0.23	0.329	0.220	0.567	0.55
Sharma	0.23	0.222	0.160	0.242	0.61	0.536
Thami	0.369	0.159	0.072	0.134	0.559	0.707
Subba	0.35	0.35	0.16	0.10	0.49	0.55
Bhutia	0.19	0.1	0.22	0.18	0.59	0.7
Lepcha	0.31	0.33	0.11	0.370	0.58	0.30
Sherpa	0.17	0.18	0.27	0.19	0.56	0.63

The variation in distribution of ABO blood group types and allele frequency in various Nepali/Gorkha sub-community (Table 4 & 5) reflects the genetic diversity which might have come from gradual migration of population thus enriching the gene pool. In the result it was seen that O blood group was seen most in Gurung, Tamang, Lama, Kami, Sherpa and Sharma. Blood group A was most frequently found in Subba, Rai, Thapa,Chhetri, Thakuri, Bhujel. Further, B type of blood group was found mostly in Damai and Sarki (Table 4).

AB was found to be the least frequent in all ethnic groups under study.

Among the Nepali/Gorkha sub-community it was seen that O blood group was most in Gurung, Tamang, Lama, Kami, Sherpa and Sharma. The Tamang, Gurung and Lama has frequency in order O>A>B>A. Blood group A was most frequently found in Subba, Rai, Thapa,Chhetri, Thakuri, Bhujel and has the phenotypic frequency in the order A>O>B>AB.

Table 5 Comparative account of the Gene frequency (p, q, r) and their distribution in hill sub- communities of Darjeeling and Kurseong

Commumity	Total Indiv.	Blood Group				Allelic Frequency			Phenotypic Frequency
Types	(N)	(A)	(B)	(AB)	(O)	(p)	(q)	(r)	
Lepcha	206	89	45	09	63	0.306	0.142	0.552	A>O>B>AB
Bhutia	160	40	49	13	58	0.181	0.217	0.602	O>B>A>AB
Nepali/ Gorkha (Sub-Comm.)	**Total Indiv.**	**Blood Group**				**Allelic Frequency**			**Phenotypic Frequency**
Bhujel	122	45	30	09	38	0.267	0.176	0.557	A>O>B>AB
Chhetri	1030	359	249	107	315	0.256	0.192	0.552	A>O>B>AB
Damai	194	46	65	20	63	0.179	0.252	0.569	B>O>A>AB
Gurung	645	170	171	67	237	0.189	0.206	0.605	O>A>B>AB
Kami	354	105	100	27	122	0.214	0.200	0.586	O>A>B>AB
Lama	297	74	57	43	123	0.171	0.186	0.643	O>A>B>AB
Mukhia	202	102	21	36	43	0.386	0.154	0.460	A>O>AB>B
Tamang	1311	409	298	112	492	0.216	0.172	0.612	O>A>B>AB
Thakuri	32	15	05	04	08	0.347	0.153	0.500	A>O>B>AB
Pradhan	644	261	159	69	155	0.314	0.197	0.489	A>B>O>AB
Rai	1958	969	346	174	469	0.368	0.144	0.488	A>O>B>AB
Thapa	536	195	137	50	154	0.271	0.194	0.535	A>O>B>AB
Sarki	44	08	18	04	14	0.144	0.293	0.563	B>O>A>AB
Sharma	251	83	54	24	90	0.232	0.170	0.598	O>A>B>AB
Thami	59	30	07	02	20	0.331	0.088	0.581	A>O>B>AB
Subba	587	282	102	56	147	0.355	0.145	0.500	A>O>B>AB
Sherpa	398	90	134	42	132	0.171	0.254	0.575	O>B>A>AB
Data collected Darjeeling	7124	2747 38.5%	1568 22.0%	696 9.76%	2113 29.66%				
Data collected Kurseong	1906	625 32.8%	479 25.1%	172 9.02%	630 33.05%				
Total Sample	9030	3372	2047	868	2743	0.272	0.178	0.550	A>O>B>AB

DISCUSSION

An ethnographical survey was made to study and analyze the ethno-demographic profile of the Hill communities residing in the hilly region of Darjeeling and Kurseong of the state of West Bengal. Reports concerning ABO blood group have been reported from different geographical, ethnic and socio-economical groups.(Beardmore, 1983).ABO blood group system is of great importance in blood transfusion and organ transplantation (Chandra and Gupta, 2012) and the susceptibility to several disease has been associated with ABO phenotypes, but though the correlation remains controversial(Anstee, 2010; Jassim, 2012).

The hill population under study comprises of multi-lingual, multi-cultural and multi-cultural composition and provides us with an interesting insight into research problems such as ancestry, population migration and evolutionary changes that may have taken place in the course of long association of different ethnic groups. Among Lepcha community the most frequent blood group was A and in the order of A>O>B>AB , Bhutia community the most frequent blood group was O and in the order of O>B>A>AB. In the Nepali/Gorkha community Group A, in the order of A>O>B>AB (Table 3). Blood group

Further, B type of blood group was found mostly in Damai and Sarki (B>O>A>AB)(Table 4)

Table 6 ABO blood group distribution in Darjeeling Hill sample and other groups of sub-Indian populations

Region	Number	A	B	O	AB
North	6334	24.7	37.50	32.50	5.30
Banjara Caste	275	22.91	37.45	27.64	12
Bangalore	36964	23.85	29.95	39.81	6.37
Thiruvanthapuram	27719	26.21	27.39	40.05	6.36
Darjeeling Hills	9030	38.55	22.01	29.66	9.76

It is evident from the different surveys in the sub-Indian populations that blood group B was most prevalent in North Indian Population.(Nanu A, Thapliyal RM.,1997) and the findings were similar to the study made by in other North Indian population, the frequency was in the order B>O>A>AB. In the South Indian population the most prevalent blood group was found to be group O as study made in Bangolore (Periyavan,s., *et al*. 2010) and Thiruvanthapuram(Abhishek B., *et al*. 2011) and was in the order O>B>A>AB Blood Group AB was found to be the least prevalent blood group.(Table 6). It is seen that in the present study in Darjeeling Hill population, the blood group A is most prevalent followed by Blood group O

and B. The frequency was in the order A>O>B>AB. The blood group AB is the least prevalent blood group.(Table 4)

The variation in the genotypic and allele frequencies of the ABO blood group system, in various Hill sub-communities reflects the genetic diversity which has come about with gradual migration. Here, it is to noted that the similarity in the genotypic and allele frequencies of some of the sub-communities suggest that these groups may have come from a common stock, like the Kirat sub-types, (Rai: p(A)= 0.368; q(B)=0.176; r(O)=0.488 and Subba: p (A)= 0.355; q(B)=0.151; r(O)=0.500 (Table 4 and Table 5) and likewise differences in the genotypic and allele frequencies suggest that these groups may have come from different ancestral stocks, for example, Rai / Subba (Kirat ethnic group) and Thakuri / Chettri / Sharma (Khas ethnic group). (Tanble 4). The genetic closeness of the different sub-communities is also reflected in the affinity it shares with regards to morphological features, customs, language and geographical distribution.

The similarities in the allele frequencies between the sub-communities like Chhetri, Sharma, Thakuri (Khas) (Table 4) suggest that they may have common from same ancestral stock. With reference to their close affinities with each other and in general to the people of Northern parts of India, these sub-communities are also referred to as Indo-Aryan race. This fact has been strongly supported by the study of historical background and migration of these races from North Western parts of India. (Pradhan, 1991). Likewise, closeness in genotypic and allele frequencies of people belonging to Dami p (A)=0.179 q(B)=0.252 r(O)=0.569 and Sarki p (A)= 0.144 q(B)=0.293 r(O)=0.563 are reflected in the physical features, culture, customs, geographical distribution and also their migration pattern.

The study of the distribution pattern of ABO blood group and allele frequencies among population of varied ethnics would be a valuable approach to plan better health care facility, provide genetic counseling and medical diagnosis of the targeted population. The present study and the findings may provide the primary insight into the indigenous Hill population of Darjeeling and Kurseong, (Table 4 & 5) for further investigation.

CONCLUSION

The ethnographic study provided an insight into the distribution pattern of ABO blood group and allele frequencies in major ethnic groups of Darjeeling and Kurseong Hills of West Bengal, India. Moreover the present data may also be useful in population genetics studies, population migration patterns, forensics and genetic studies and genetic counseling.

References

1. Abhishek B, Maya devi S, Meena D, Usha KC. Distribution of ABO and Rhesus-D blood groups in and around Thiruvnthapuram. Kerala Med J. 2011;1:28-9.
2. Anstee D.J,The relationship between blood groups and disease. Blood 2010; 115(23):4635-4643.
3. Beardmore J and Karimi-Booshehri F. ABO genes are differentially distributed in socio-economical groups in England. Nature 1983;303:522-524.
4. Chandra. T and Gupta A. Association and distribution of Hypertension, Obesity and ABO Blood groups in Blood Donors. *Iranian journal of Pediatric Hematology Oncology* 2012; 2(4):140-145.
5. Ghobadian Z, Sayemiri K,Zeinali M, Sajjadi SM. Distribution of ABO and Rh blood groups in a major ethnic group of the West Iran, the Kurdish population. *Asian Journal of Medical Sciences* 2013;5(3):26-29.
6. Jassim W. Association of ABO blood group in Iraqis with hypercholesterolemia, hypertension and diabetes mellitus. EMHJ 2012;18(8):888-889.
7. Mckusick, V.A. (1978) Human Genetics (2e) Prentice Hall of India, New Delhi.
8. Miller JZ, Grim CE, Conneally P and Weinberger MH. Association of Blood groups with essential and secondary hypertension. A possible association of the MNS system. Hypertension 1979;1(5):493-497
9. Mondal B, Maiti S, Biswas BK, Ghosh D and Paul S. Prevelence of hemoglobinopathy, ABO and rhesus blood groups in rural areas of West Bengal, India. *Journal of Research in Medical Sciences* 2012; 17(8):772-776.
10. Nanu A, Thapliyal RM. Blood group gene frequency in a selected north Indian population. *Indian J Med Res.* 1997; 106:242-6.
11. Pennap G, Envoh E and Igbawua I, Frequency Distribution of Hemoglobin Variants, ABO and Rhesus Blood groups among Students of African Descent. *British Microbiology Research Journal* 2011; 1 (2):33-40.Anstee D.J, The relationship between blood groups and disease. Blood 2010; 115(23):4635-4643.
12. Periyavan S, Sangeetha SK, Marimuthu P, Manjunath BK, Seema DM. Distribution of ABO and Rhesus-D blood groups in and around Bangalore. *Asian J Transfus Sci.* 2010;4:41.
13. Pradhan, K. (1991) The Gorkha Conquests, Oxlord University Press.
14. Raghu. P, A.Deva, R. Nandakumar, S. Ganga and k. Prabhakaran. ABO Blood group polymorphisms in eleven Tribal populations of South India. *World Journal of Pharmaceutical Research* 2016:5(3): 1826-1836.
15. Singh, K.S.(1993) People of India Sikkim, Volume XXXIX, Anthropological Survey of India. Seagull Books, Calcutta.